Hypoxia Driven Maturin Expression in Ovarian Carcinoma Cell Lines

Peter Wilkinson, Vivek Ramakrishnan, Erica Schnettler, Sundaram Ramakrishnan
Department of Pharmacology, University of Minnesota, Minneapolis, MN, USA.

University of Minnesota
Driven to Discover

Abstract

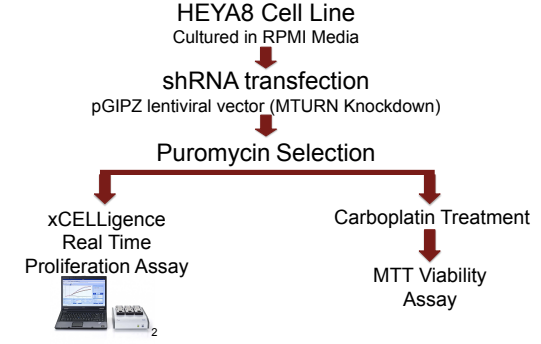
Ovarian carcinoma is a highly metastatic cancer that is often diagnosed at an advanced stage. As ovarian cancer develops it metastasizes into the peritoneal cavity, where tumors invade the mesothelium.[1] Here tumors encounter a hypoxic microenvironment due to irregular vascularization and stagnant ascites fluid. Microenvironment stress promotes tumors to initiate angiogenesis, the formation of new blood vessels, in order to access oxygen and nutrients from the blood. Further understanding hypoxia-induced angiogenesis is critical for improving patient treatment and identifying novel pharmacological targets. Previous work in our laboratory has identified the Maturin protein as a target of interest in the hypoxic response of ovarian carcinoma. Maturin is translated from a highly conserved gene named MTURN. The protein is uncharacterized in ovarian carcinoma. We hypothesize that Maturin is involved in the hypoxic response of ovarian carcinoma. To test the hypothesis we quantified MTURN expression under hypoxic stress in A2780, HEYA8, and OVCAR3 cell lines. Furthermore, the Maturin phenotype was investigated in vitro using an shRNA knockdown vector in HEYA8 cells. Our results indicate that MURN expression is up regulated in hypoxia, in vitro knockdown of the gene modulates cell proliferation and sensitizes cells to carboplatin treatment.

Methods

Fig 1: Ovarian Carcinoma Cell Lines (A2780, HEYA8, OVCAR3) → Hypoxia Exposure → quantitative PCR

Experimental Technique: 95% N_2 + 5% CO_2 Exposure → 24 hour Incubation

Fig 2:



Results

MTURN Expression in Hypoxia

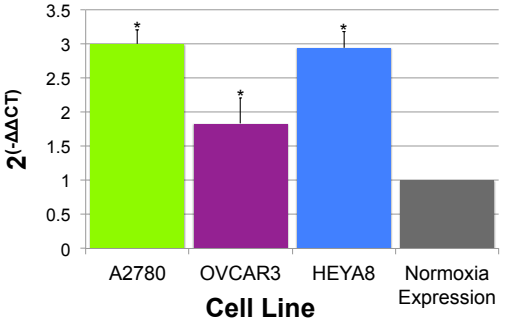


Fig 3: A2780, OVCAR3, and HEYA8 cells lines were incubated in a hypoxia chamber for 24 hours. RNA was extracted via a Trizol RNA extraction. Quantitative PCR was performed with the hypoxic RNA compared to RNA extracted from cells cultured in normoxia. Beta actin primers were used as an endogenous positive control. The experiment was repeated three times. * $p < 0.05$

MTURN Knockdown Carboplatin Sensitivity in HEYA8 Cells

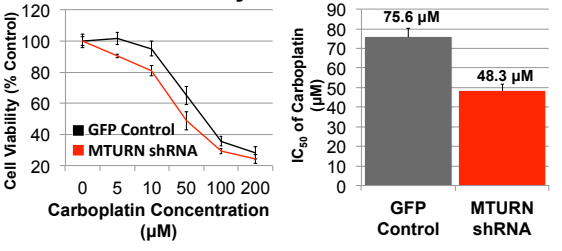


Fig 4: Transfected HEYA8 cells were plated in a 96 well plate at 5000cells/well in 100μl of RPMI media. Cells were incubated 12 hours at 37°C in 5% CO_2 and treated with carboplatin in doses ranging from 1-200μM. After treatment cells were incubated for 48 hours. A MTT Assay was performed and the 590nm absorbance was measured using a plate reader. IC_{50} of carboplatin concentration was calculated from the concentration response curve.

Real Time Proliferation of MTURN Knockdown

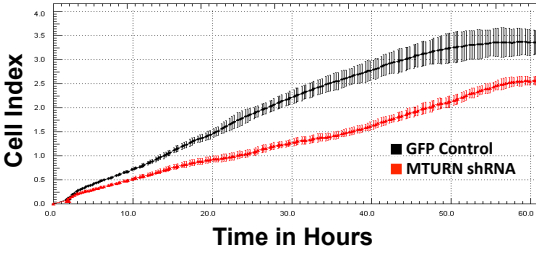


Fig 5: A real time proliferation assay was performed using the xCELLigence system. Transfected HEYA8 cells were plated at 5000 cells/well in xCELLigence e-plates in 100μl of RPMI media. Proliferation was measured in the system for 72 hours at 37°C in 5% CO_2. The experiment was repeated three times. Data from a representative experiment.

Conclusions

- MTURN expression is up regulated in response to hypoxia in HEYA8, A2780, and OVCAR3 cell lines
- MTURN knockdown via shRNA reduces HEYA8 proliferation rate in vitro
- MTURN knockdown via shRNA sensitizes HEYA8 cells to carboplatin treatment

Future Studies

- Effect of MTURN knockdown on cell cycle
- Western blot protein expression verification
- Characterization of MTURN expression in mouse tumor models
- Further drug sensitization screening

References & Acknowledgement

1. Lengyel, E. (2010). Ovarian Cancer Development and Metastasis. *The American Journal of Pathology*, *177*(3), 1053–1064. doi:10.2353/ajpath.2010.100105
2. XCELLigence DP Real Time Cell Analyzer. Westburg BV. Web. 2016.
3. Supported by U.R.O.P.